

Application No. 09/856,339  
Amendment dated November 29, 2005  
Reply to Office Action of June 29, 2005

Docket No.: HO-P02196US0

**a) Amendments to Claims**

As of the office action of June 29, 2005, claims 2-3, 5, and 22-25 were pending. In this response, claim 2 is canceled, and claims 3, 5, and 25 are amended. As of this response, claims 3, 5, and 22-25 are pending.

1.-2. (Canceled).

3. (Currently Amended) The process according to claim ~~[[2]]~~ 25 in which the enzyme is one in which at least amino acid 47 and/or 51 of P450<sub>BM-3</sub>, or amino acid 96 of P450<sub>cam</sub>, have been changed to an amino acid with a less polar side-chain.

4. (Canceled).

5. (Currently Amended) The process according to claim 25 in which the enzyme is (i) a P450<sub>cam</sub> mutant enzyme and comprises at least one or more of the following mutations: F87W, F87L, F87L, T185L, T185F, V247A, V247L or F87A I395F; or (ii) P450<sub>BM-3</sub> and comprises the mutation R47L-Y51F V247A or V247L; (ii) a P450<sub>cam</sub> mutant enzyme and comprises at least both the mutations F87A and I395F; or (iii) a P450<sub>BM-3</sub> mutant enzyme and comprises at least both the mutation R47L and Y51F.

6.-21. (Canceled).

22. (Previously Presented) The process according to claim 25 in which the substrate is selected from the group consisting of limonene, pinene, and substituted derivatives thereof.

23. (Previously Presented) The process according to claim 25 in which the substrate is a cyclic sesquiterpene, or a substituted derivative thereof.

24. (Previously Presented) The process according to claim 23 in which the substrate is selected from the group consisting of aromadendrene, carophyllene, longifolene, valencene,

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isobazzanene, silphinene, ishwarane, isopatchchoul-3-ene, isosesquicarene, and substituted derivatives thereof.

25. (Currently Amended) A process for oxidising a substrate wherein said substrate is a limonene or pinene or a cyclic sesquiterpene, or a substituted derivative thereof, wherein the substituent is an alkyl of 1 to 6 carbons or an alkenyl of 1 to 6 carbons and wherein the process comprises: oxidizing said substrate with a mutant haem-containing enzyme, with the proviso that:

~~the substituent is not a halogen, and~~

~~the substituent does not comprise an oxygen atom,~~

~~and wherein the process comprises: oxidizing said substrate with a mutant haem-containing enzyme,~~

wherein when the substrate is a limonene or a pinene or substituted derivative thereof, the enzyme is selected from the group consisting of:

(a) a P450<sub>cam</sub> variant mutant enzyme corresponding to of SEQ ID NO:1, comprising a mutation [[at]] of at least two or more of the following amino acid positions: 87, 96, 244, 247, or 248, wherein the P450<sub>cam</sub> mutant enzyme has a higher oxidation activity towards the substrate which is being oxidised than an enzyme having the sequence SEQ ID NO:1, and wherein at least one of said mutations is a substitution of an amino acid by an amino acid with a less polar side-chain;

~~(b) a P450 monooxygenase homologue variant enzyme with at least 95% sequence homology with SEQ ID NO:1 comprising at least two or more mutations at amino acid positions which are equivalent to amino acid positions 87, 96, 244, 247, or 248, of P450<sub>cam</sub> enzyme corresponding to SEQ ID NO:1;~~

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(e) ~~(b)~~ a P450<sub>BM-3</sub> variant mutant enzyme ~~corresponding to~~ of SEQ ID NO:24, comprising a mutation ~~[[at]]~~ of at least one or more of the following amino acid positions: 47, 51, or 87, wherein the P450<sub>BM-3</sub> mutant enzyme has a higher oxidation activity towards the substrate which is being oxidised than an enzyme having the sequence SEQ ID NO:24, and wherein at least one of said mutations is a substitution of an amino acid by an amino acid with a less polar side-chain; and,

~~(d) a P450 monooxygenase homologue variant enzyme with at least 95% sequence homology with SEQ ID NO:24 comprising at least one or more mutations at amino acid positions which are equivalent to amino acid positions 47, 51, or 87 of P450<sub>BM-3</sub> enzyme corresponding to SEQ ID NO:24;~~

and wherein when the substrate is a cyclic sesquiterpene or substituted derivative thereof, the enzyme is selected from the group consisting of:

(e) ~~(c)~~ a P450<sub>cam</sub> variant mutant enzyme ~~corresponding to~~ of SEQ ID NO:1 comprising a mutation of at least two or more of the following amino acid positions: 87, 96, or 244, wherein the P450<sub>cam</sub> mutant enzyme has a higher oxidation activity towards the substrate which is being oxidised than an enzyme having the sequence SEQ ID NO:2, and wherein at least one of said mutations is a substitution of an amino acid by an amino acid with a less polar side-chain;

~~(f) a P450 monooxygenase homologue variant enzyme with at least 95% sequence homology with SEQ ID NO:1 comprising at least two or more mutations at amino acid positions 87, 96, or 244;~~

(g) ~~(d)~~ a P450<sub>BM-3</sub> variant mutant enzyme ~~corresponding to~~ of SEQ ID NO:24, comprising a mutation ~~[[at]]~~ of at least one or more of the following amino acid positions: 47, 51, or 87, wherein the P450<sub>BM-3</sub> mutant enzyme has a higher oxidation activity towards the substrate

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which is being oxidised than an enzyme having the sequence SEQ ID NO:24, and wherein at least one of said mutations is a substitution of an amino acid by an amino acid with a less polar side-chain.[:]] and,  
~~(h) a P450 monooxygenase homologue variant enzyme with at least 95% sequence homology with SEQ ID NO:24 comprising at least one or more mutations at amino acid positions which are equivalent to amino acid positions 47, 51, or 87 of P450<sub>BM3</sub> enzyme corresponding to SEQ ID NO:24;~~

~~wherein said mutations in (a), (b), (c), (d), (e), (f), (g), and (h) are a substitution of an amino acid by an amino acid with a less polar side-chain.~~